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Phytochemical and pharmacological analysis of Pterodon polygalaeflorus extracts

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Abstract

Fruits from *Pterodon* species are commercially available at medicinal flora markets, being largely used in folk medicine by its anti-arthritic, analgesic and anti-inflammatory actions. This study compared the antinociceptive and anti-inflammatory potentials of the ethanol (EEPpg), hexane (HEPpg) and dichloromethane (DEPpg) extracts from *Pterodon polygalaeflorus* Benth. fruits. Inhibition of nitrite production by RAW 264.7 cells, determined with Griess reagent, presented IC_{50} values of 11.93 µg/ml (EEPpg), 6.02 µg/ml (HEPpg) and 11.86 µg/ml (DEPpg). In the air pouch model, all extracts reduced the total number of leukocytes in the exudate at all tested doses, in relation to control group, the highest inhibition being observed with HEPpg (62.3%). Macroscopic analysis of the pouch tissues showed reduction of inflammatory signs (redness, vasodilation) on treated mice, mainly with HEPpg, which also reduced microscopically the tissue thickening and leukocyte infiltration. The highest antinociceptive potential (100%) in the writhing test was observed with HEPpg. Phytochemical analysis identified 90% of extract's content, composed by sesquiterpene hydrocarbons, oxygenated sesquiterpenes and furan diterpenes. HEPpg was the extract with the highest anti-inflammatory and antinociceptive actions and comparison with literature data suggests a synergy of compounds due to higher antinociceptive activity with a lower dose than that observed with isolated vouacapan derivatives.

KEY WORDS: PTERODON POLYGALAEFLORUS, ANTINOCICEPTIVE ACTIVITY, AIR POUCH MODEL, NITRIC OXIDE PRODUCTION, PHYTOCHEMISTRY

Introduction

Inflammation is a body physiological response to physical damage, microorganism invasion or immune response to antigens or irritating chemicals [1]. When the inflammation regulatory mechanisms are lost, the response becomes pathological, giving rise to several diseases such as osteoarthritis, multiple sclerosis, psoriasis and chronic asthma [2, 3]. Many of these diseases are debilitating and increasingly common in the current aging society. Nonsteroidal anti-inflammatory drugs and glucocorticoids are among the various therapeutic approaches currently used for the treatment of inflammatory diseases, and several side effects have been associated with their prolonged use [4]. This way, the World Health Organization (WHO) has been encouraging the study of traditional plants since 1977, which represents an important source of bioactive substances. There are over 500,000 species worldwide, with approximately 120 thousand of them found in Brazil [5], many without scientific study of their properties.

The genus *Pterodon* (Leguminosae, Fabaceae) comprises five species native to Brazil: *P. abruptus* Benth., *P. apparicioi* Pedersoli, *P. polygalaeflorus* Benth., *P. emarginatus* Vog. and *P. pubescens* Benth., popularly known as sucupira branca. The fruits from *Pterodon* species are commercially available at the medicinal flora market, being largely used in folk medicine to treat rheumatism, arthritis and by its analgesics and anti-inflammatory properties [6, 7].

Data from the literature show that the essential oil of *Pterodon polygalaeflorus* Benth fruit modulates acute inflammation and lymphocyte activation [8] and that the hydroalcoholic extract presents antinociceptive effects [9]. Otherwise, higher anti-inflammatory [10] and antinociceptive [11] effects have been demonstrated for *P. pubescens* samples obtained with solvents of different polarities. The hexane subfraction showed higher acute and topic anti-edematogenic potential than the original ethanol extract (OEP) [10, 12], and the evaluated antinociceptive property of *Pterodon pubescens* was also increased after subfractionation with dichloromethane [11], instead of hexane fraction. In addition, the hydroalcoholic extract of *P. pubescens* seeds (HEPp) presented anti-arthritic [13, 14] and immunosuppressive activity [15].

Based on the diversified studies demonstrated for Pterodon species and on the findings of different intensities of the observed effects, depending upon the extraction procedure, in this study, we compared the chemical composition and the antinociceptive and the anti-inflammatory activities between the ethanol (EEPpg), hexane (HEPPg) and dichloromethane (DEPpg) extracts from *Pterodon polygalaeflorus* fruits.

Material and Methods

Plant material and extraction

The taxonomic identity of *Pterodon polygalaeflorus* Benth. (Leguminosae/Fabacea) was confirmed by Haroldo Cavalcante de Lima, from the Department of Systematic Botany, Jardim Botânico do Estado do Rio de Janeiro, Brazil, where a voucher specimen has been deposited (RB 350278, July 1999). *Pterodon polygalaeflorus* fruits were powdered in liquid nitrogen and submitted to maceration (10 ml/g fruit) with ethanol, hexane or dichloromethane, at room temperature for 15 days. Following solvent evaporation (rotary evaporator) the ethanol (EEPpg), hexane (HEPpg) and dichloromethane (DEPpg) extracts were obtained yielding (w/w) 33.9%, 32% and 39%, respectively.

To *in vitro* assays extract samples were dissolved in ethanol and then diluted with a supplemented medium consisting of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FCS) (Cultilab, Brazil), penicillin (70 mg/l) and streptomycin (100 mg/l) to a final ethanol concentration of 0.16%. To chromatographic analysis, samples are dissolved in methanol at 10 mg/ml and for *in vivo* assays extracts were prepared with 15% ethanol 1.25% Tween-20 (vehicle). Control experiments did not show an altered response due to oral administration of the vehicle in the concentration used [11].

Animals

Male Swiss Webster (SW) mice with 25-35 g body weight (b.w.) were kept at 25°C in 12 h light-dark cycle and maintained with water and food *ad libitum*. For each experiment, mice were randomly selected into groups comprising five per cage. Studies were carried out in accordance with current guidelines on the care of laboratory animals and ethical guidelines on the investigation of experimental pain in conscious animals [16] and under the consent and surveillance of the Ethics Committee for Animal Research of the Institute of Biology of the State University of Rio de Janeiro (CEA-IBRAG committee/protocol 05/2009 and 007/2013).

Chemicals

Acetylsalicylic acid (ASA) was acquired from Merck, Brazil. Carrageenan λ, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), ribonuclease A, indomethacin, **Dulbecco's Modified Eagle's Medium** (**DMEM)**, sodium dodecyl sulfate (SDS), Tween 20, were purchased from Sigma Chemical Co., St Louis, MO, USA and fetal bovine serum (FBS) from Vitrocell, Brazil. HPLC grade N-hexane 95%, ethyl acetate, ethanol 100% and dichloromethane were purchased from Tedia, Brazil and purified paraffin from Proquímios, Brazil. All other chemicals and reagents used were high-grade purity.

Cell line

Mouse macrophage cell line RAW264.7 obtained from Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil, Oct 2000) have been cryopreserved in liquid nitrogen and used for the *in vitro* analysis. This cell line originated from a tumor induced in BALBc mice by the Abelson murine leukemia virus, being negative for immunoglobulin expression and Thy 1.2 on its surface. For the experiments, the cells were cultured in a supplemented medium (DMEM with 10% FBS) at 37°C and 5% CO_2 humid atmosphere being the passages held every 48 h.

MTT Cytotoxicity assay

RAW 264.7 cells (5 x 10^4 cells/100 µl/well) were cultured (triplicates) in 96 flat-bottom-well plates (Becton Dickinson Labware, USA) for 24 h at 37°C, with 5% CO_2 to the cells' adherence. The cells' were then incubated with different concentrations of samples (EEPpg, HEPpg, DEPpg) for 24 h at 37°C and 5% CO_2. The cells survival was determined by the MTT assay [17] adding 10 µl/well of stock solution of MTT 5 mg/ml in phosphate-buffered saline pH 7.4 (PBS). Following incubation for 2 h, 100 ìl/well of SDS 10% solution in 0.01 N HCl was added. The extent of formazan crystal production was measured by absorbance at 570 nm (microplate reader ìQuant, Bio-Tek Instruments, Inc.). Extract/fraction's samples were not added to the control cultures.

No cytotoxicity was observed with control cultures that received only 0.016% ethanol in the supplemented medium (diluent of samples). The results are expressed as percentage of mitochondrial reducing activity (MRA) in relation to control group (absorbance value considered as 100%).

Nitrite assay

Nitrite, a stable product of nitric oxide (NO) in aqueous solutions was measured in the incubation medium with Griess reagent [18]. RAW 264.7 cells (5 x 10^4 cells/100 µl/well) were cultured (triplicates) in 96 flat-bottom-well plates (Becton Dickinson Labware, USA) for 24 h at 37°C, with 5% CO_2 to the cell's adherence. After this, the cells were incubated for 24 h with LPS (1 µg/ml) in the absence or presence of different concentrations of samples (EEPpg, DEPpg and HEPpg) at 37°C with 5% CO2. Then, 25 µl of the supernatant of each well was transferred to another plate, 25 µl of sulphanilic acid (10 mg/ml) were added, and the plate was incubated for 10 min in the dark. Thereafter, 50 µl/well of N-(1-naphthyl) ethylenediamine (1 mg/ml) were added, the plate was incubated for 10 min and finally, the absorbance was read at 550 nm (microplate reader µQuant, Bio-Tek Instruments, Inc.). The

results were expressed as nitrite concentration obtained from the standard curve built using a solution of sodium nitrite or as the percentage of inhibition of nitrite production relative to the control.

Air pouch model

The pouch was created by a sterile air injection of 5 ml in the mouse back and maintained by 3 ml injection of sterile air three days after the first one [19, 20]. In the sixth day, one hour before the administration of 1 ml carrageenan 1% into the cavity, different doses of extracts (100 ìl) prepared with the vehicle were orally administrated. One group received the vehicle (control), and another was treated with the control drug Indomethacin (Indo, 10 mg/kg b.w.). Four hours after carrageenan injection, the animals were killed in a CO_2 chamber; air-pouch was injected with 1.0 ml of ice-cold physiological saline with 2 mM EDTA; their cavities were exposed; the exudates were collected; the volumes were measured, and the total number of leukocytes determined using a hemocytometer. The air pouch tissues were removed, photographed and processed for histological analysis.

Acetic acid-induced abdominal constriction

Mice were pre-treated with extracts or 100 mg/kg ASA (p.o.) 60 or 30 min, respectively, before intraperitoneal (i.p.) injection of 0.6% AcAc (10 ml/kg) [21]. The nociception control group received, p.o., equal volume of the vehicle. Control experiments did not show an altered response due to oral administration of the vehicle in this concentration. The number of writhes, defined as a sequence beginning with arching of the back, contraction of the abdomen, twisting of the trunk and/or pelvis, and usually ending in the extension of the limbs, were observed for 10 min beginning 5 min after acetic acid injection [22]. Antinociceptive activity was detected as a reduction of the writhing number exhibited by treated mice compared with the control group (% pain inhibition).

Gas chromatography-mass spectrometry

Extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). Qualitative analysis were conducted in a GC-MS QP2010 Plus Shimadzu (70 eV) apparatus with a Rtx®- 5MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm). The operating temperatures were as follows: injector 260°C, detector 200°C and column oven 50°C up to 290°C (3°C/min). Helium at 1 ml/min was used as a carrier gas. Mass spectral fragmentations obtained were compared to the data bank of National Institute of Standards and Technology (NIST 05) library and with the literature [23-27].

Statistical analysis

Results are presented as mean values ± S.D. Data were subjected to analysis of variance (One-way ANOVA) followed by Tukey's or Dunnet test, as appropriate, using GraphPad Prism 5. Differences between groups were considered significant at a level of $p \leq 0.05$. IC_{50} values (concentration that reduces 50% of control group response) were determined by non-linear regression.

Results

Effect of *P. polygalaeflorus* extracts on cytotoxicity and on nitric oxide production by RAW 264.7 cells

The NO production by RAW 264.7 cells was stimulated with LPS for 24 h, showing 29.7± 3.0 µM (Figure 1 A-C). All extracts presented significant inhibitory action on this inflammatory mediator production. EEPpg (Figure 1A) significantly reduced the NO production by 41.0%, 69.7%, 95.0 % and 91.3% at 10, 20, 40 and 80 µg/ml, respectively. DEPpg (Figure 1B) also inhibited the NO production by 40.2%, 69.2%, 88.5% and 93.6%, at same concentrations. HEPpg (Figure 1C) significantly inhibited the production of nitrite by 35.7%, 50.2%, 64.3%, 92.7% and 97.1%, at concentrations of 1, 10, 20, 40 and 80 µg/ml, respectively.

The concentration of EEPpg, HEPpg and DEPpg which reduces 50% of NO production (IC_{50}) were 11.93 µg/ml, 6.02 µg/ml and 11.86 µg/ml, respectively. In order to assess whether the inhibition of nitrite production was correlated with the extract's cytotoxicity, the RAW 264.7 cells viability was assessed with different concentrations of each extract (Figure 1 D-F). EEPpg (Figure 1D) DEPpg (Figure 1E) and HEPpg (Figure 1F) showed a significant decrease of the MRA, mainly at 40 µg/ml, with viability inhibition indexes of 42.6%, 46.9%, and 41.5%, respectively.

Despite the presence of cytotoxic effects of both extracts at 40 µg/ml, this concentration and lowest ones have induced higher inhibition levels on NO production than on the cell viability, which strongly suggest that are associated with the anti-inflammatory potential of extracts.

see Figure 1

Evaluation of anti-inflammatory potential of extracts

The total number of leukocytes that migrated to the inflammatory cavity was determined. Carrageenan injection induced inflammatory cell migration to the air pouch cavity in the control group, compared with the saline group (Figure 2). Treatment with the EEPpg (Figure 2A), DEPpg (Figure 2B) or HEPpg (Figure 2C) reduced the total number of leukocytes in the exudate at all tested doses when compared with the control group. The highest inhibition index was observed with HEPpg treatment, which reduced between 51.8% and 62.3% the cell migration to the air pouch. Indomethacin reduced 69.7 ± 3.4% of cell migration into the cavity.

Macroscopic analysis of morphological changes within the pouch tissues of animals treated with both extracts were then performed and compared to the control group. Figure 3 shows the photographic images of the tissue pouch from representative animals from each group. The control group, treated with vehicle (Figure 3B), showed inflammatory signs as redness, vasodilation and/or formation of new blood vessels, which was not exhibited by the group of animals inoculated only with saline (Figure 3A). Groups inoculated with carrageenan and treated with indomethacin, as control drug (Figure 3F), or with 1 mg/kg EEPpg (Figure 3C), DEPpg (Figure 3D) or HEPpg (Figure 3E) showed visible reduction of inflammatory patterns (vasodilation and redness), mainly the HEPpg, whose histological changes were also evaluated (Figure 4).

Tissue from the control group with the vehicle (Figure 4B) exhibited an accumulation of leukocytes in the deepest layer of the pouch in relation to the saline group (Figure 4A). Treatment of animals with HEPpg inhibited the inflammatory cell infiltration into the deepest layer of tissue and reduced the tissue thickening (Figure 4D), compared with the vehicle control group (Figure 4B). The anti-inflammatory signs found in the HEPpg histological studies were similar to that observed with the traditional anti-inflammatory indomethacin drug (Figure 4C).

see Figure 2, 3 and 4

Evaluation of antinociceptive potential of extracts

The screening of antinociceptive activity of extracts was performed by the abdominal constrictions' model (Table 1). The control drug AAS presented maximal writhing inhibition of 45%. The lowest inhibition was observed with EEPpg (between 22.2% and 47.4%). The writing inhibition by the DEPpg remained between 40.9% and 71.9%, and the HEPpg inhibited 100% of abdominal constrictions at 0.5 and 1.0 mg/kg doses.

see Table 1

Phytochemical analysis

By GC-MS, 17 components were identified in the extracts (Table 2) representing about 90% of the total content of extracts; however, only nine compounds were found in all three extracts (β-elemene, β-caryophyllene, β-humulene, farnesol,

6α-hydroxy-7β-acetoxy vouacapan-14(17)-ene, methyl-6α,7β-dihydroxy vouacapan-17β-oate, 6α-acetoxy-7β-hydroxy vouacapan, methyl-7β-acetoxy-6α-hydroxyvouacapan-17β-oate and methyl-6α-acetoxy-7β-hydroxyvouacapan-17β-oate) and at different relative concentrations.

The extracts are composed mainly by sesquiterpene hydrocarbons, oxygenated sesquiterpenes and furan diterpenes. Sesquiterpenes from HEPpg are mainly β-elemene, β-caryophyllene, spathulenol, caryophyllene oxide and farnesol (46.08%). Sesquiterpenes from DEPpg and EEPpg are mainly β-elemene, β-caryophyllene and germacrene D, which constituted 34.88% and 39.97% of total compounds, respectively. Furan diterpenes with vouacapan group are present in HEPpg, DEPpg and EEPpg extracts, constituting 39.36%, 49.10% and 41.73% of the compounds, respectively.

see Table 2

Discussion

The inflammatory response is a defense mechanism evoked by body tissues in response to injury or microbial invasion and is characterized by redness, heat, pain and swelling [28]. During this process, pro-inflammatory mechanisms are offsetted by anti-inflammatory regulatory pathways, making it self-limiting. However, many diseases can be developed from or accompanied by excessive inflammatory processes, and can constitute, on its own a source of discomfort thus requiring treatment. The medicinal plants owe their anti-inflammatory activities to their phytochemical constituents, interfering at a different level on the response, such as diminishing pro-inflammatory mediator's release (*e.g.* cytokines, NO, bradykinin), inhibiting complement activation or reducing leukocyte migration to inflammation sites, etc. [29, 30].

We, firstly, compared the extract effects on the NO production by RAW264.7 cells and their respective cytotoxicities. NO is a low-molecular-mass soluble mediator involved in many physiological processes and produced, during inflammation, by activated inflammatory cells, especially macrophages and neutrophils. The NO pro-inflammatory activity involves pro-oxidant action and vasodilation, important feature for leukocyte migration to the inflammatory site. Excessive NO production promotes DNA damage, protein structure changes, cellular function alteration, mitochondrial respiration dysfunction and cell death, resulting in disease's development and organ failing [31, 32]. Therefore, NO is one of the several targets for development of complementary and alternative medicines to treat chronic inflammatory diseases and cancer [33].

In this work, the NO production by RAW 264.7 macrophages was stimulated by the *E. coli* Lipopolysaccharide (LPS) [34, 35] and this result showed a significant increase in NO production, according to literature data [36]. All extracts showed significant anti-inflammatory potential, reducing the *in vitro* production of this vasodilatation mediator. However, comparatively, the EHPpg showed a higher inhibitory effect than the other extracts, presenting the smallest IC_{50}. The extracts showed very similar cytotoxic potential, but it is important to note that significant inhibition indexes on NO production, at lower HEPPg concentrations induced low cytotoxic effects. The discovery of NO inhibitors, thus, may represent a significant therapeutic advance for inflammatory diseases. Our results are in agreement with those from other groups that also exhibited inhibition of NO production by another plant extract as a mechanism of its anti-inflammatory activity [36, 37].

The anti-inflammatory effects of the *Pterodon polygalaeflorus* extracts were also compared using an *in vivo* inflammatory response, the air pouch model, described by Sedgwick *et al.* [38]. The air cavity, induced by sterile air injection on the back of rodents, works as an exudate reservoir containing cells and mediators, which can be collected and analyzed [39]. The introduction of carrageenan in this cavity induced an inflammatory response in the vehicle group, showing a high number of inflammatory cell infiltrated (Figure 2), according to the literature [19], vasodilatation, redness and fibrin

deposition (Figure 3), and leukocyte infiltration in the deepest layer of air pouch tissue (Figure 4), as compared with the saline group. These aspects of inflammatory response are similar to those of chronic inflammation in a rheumatoid joint [40]. As blood neutrophils represent the first cells to migrate to the inflammatory site [41], these cells represent the major cell type in the air pouch cavity of the vehicle group. HEPpg was the most effective extract in the *in vivo* model, showing the greater inhibitory index (62.3%) of leukocyte migration to the inflammatory cavity, at all concentrations tested, similar to that observed by Indomethacin (69.7%) group (Figure 2). Treatment with 1 mg/kg HEPpg also reduced the vasodilation and redness (Figure 3) and the tissue inflammatory infiltrate (Figure 4).

Therefore, it may be suggested that the decrease of the NO production, with consequently, reduced vasodilation and blood vessel permeability, may be contributing to the inhibition of leukocyte migration to the inflammatory site. Besides, modification in the activation level of adhesion molecules in leukocytes or endothelial cells, reduction of pro-inflammatory cytokines or increase of anti-inflammatory cytokines may also be contributing to the anti-inflammatory action of *P. polygalaeflorus* extracts.

In mammals, potentially aggressive stimuli coming from the external environment are detected by specialized structures in the peripheral nervous system, called nociceptors. An injured or inflamed tissue generates numerous molecules activating nociceptors [42]. The antinociceptive properties of different extracts were evaluated by the writhing test because it has been widely used as a screening tool for search of analgesic or anti-inflammatory new agents [43-45]. The acetic acid in peritoneal cavity causes local irritation and triggers the release of several mediators such as bradykinin (BK), substance P (SP), prostaglandins (PGs), and also the release of some cytokines such as TNF-α, IL-β 1 and IL-8, by peritoneal residents macrophages [46, 47]. The number of abdominal contortions was inhibited by all extracts of *P. polygalaeflorus*, with HEPpg exhibiting the higher antinociceptive effect (100% inhibition of abdominal constrictions). These results suggest that the antinociceptive effect here observe may be related to inhibition of pro-inflammatory mediators or both types of mediators, whose production is induced by acetic acid.

Different pharmacological studies with *Pterodon* species were developed with hydroalcoholic [9, 13], ethanol [25, 26, 48-51], petroleum ether [52, 53], acetone [54], hexane [48, 55-60], and dichloromethane extracts [27], as well as with essential oil [61-63]. Phytochemical analysis of *Pterodon* species has shown the presence of diterpenes [24, 59] and isoflavones [64] in fruit oil. Furan diterpenes with several oxidation's patterns on the vouacapan skeletons were also identified and isolated from *Pterodon* species [24, 48, 53, 59, 65], and their anti-inflammatory, antinociceptive and antiproliferative properties [10, 11, 58, 60, 65-69,] have been attributed to these substances.

In this work, the phytochemical analysis of the extracts showed the vouacapan derivatives 6α-hydroxy-7β-acetoxyvouacapan-14(17)-ene; methyl-6α,7β-dihydroxy vouacapan-17β-oate; 6α-acetoxy-7β-hydroxy vouacapan; 6α,7β-diacetoxy vouacapan; methyl-7β-acetoxy-6α-hydroxy vouacapan-17β-oate and methyl-6α-acetoxy-7β-hydroxy vouacapan-17β-oate, which performed a total of 39.36%, 49.10% and 41.73% of compounds present in HEPpg, DEPpg and EEPpg, respectively. Sesquiterpenes were also present in these extracts performing 50.97%, 42.73% and 44.93% of compounds present in HEPpg, DEPpg and EEPpg, respectively. Sesquiterpenes from HEPpg are mainly β-caryophyllene (10.1%), β-elemene (14.01%) and caryophyllene oxide (12.21%), while from DEPpg are mainly β-caryophyllene (10.7%), β-elemene (15.93%) and germacrene D (8.78%) and from EEPpg are mainly β-caryophyllene (13.42%), β-elemene (15.65%) and germacrene D (10.05%).

Although antinociceptive and anti-inflammatory activities of Pterodon species have been commonly assigned to the vouacapan derivatives, many pharmacological activities have been demonstrated

for sesquiterpenes, which were also described in these extracts. Beta-elemene, present in HEPPg, DEPpg and EEPpg, protected endothelial cells from injury induced by hydrogen peroxide [70], suppressed VEGF-mediated angiogenesis [71] and presents antitumor activity in a broad range of human cancer cell lines, which is associated with both cell cycle arrest and apoptosis induction [72, 73]. Beta-caryophyllene, identified in all three extracts exhibits anti-inflammatory [74], anticarcinogenic [75], antibiotic [76], antioxidant [77], local anesthetic [78] activities, as well as antispasmodic effect in an isolated rat ileum [79].

Some compounds preferentially found in HEPpg, also show anti-inflammatory and/or antinociceptive action. Caryophyllene oxide presented central and peripheral analgesic activity and anti-inflammatory action [80]. Alfa-humulene exhibited marked anti-inflammatory properties [81], while immunomodulatory activities have been attributed to spathulenol, also described in *Salvia mirzayanii* [82]. Finally, the isoprenoids farnesyl acetate inhibits DNA replication in HeLa cells [83] and farnesol have been shown antioxidant, anti-inflammatory [84] and antinociceptive effects [85].

The highest *in vitro* and *in vivo* pharmacological activities were observed with the HEPpg, which also presented the lowest content of vouacapan derivatives and the highest concentration of sesquiterpenes.

Literature data with the writing test shows that 6a-7b-dihydroxy vouacapan-17b-oate at 185 mg/kg produced 91% inhibition by i.p. route [67], 61% inhibition by s.c. route and 46% inhibition by oral administration [86]. Also, 6α,7β-dihydroxyvouacapan-17β-oate methyl ester at 30 mg and 300 mg/kg (i.p.) inhibited 88% and 98% of writhes, respectively [69, 87]. The 6a,7 β-dihydroxy-vouacapan-17b-oic acid (i.p.) inhibited 48% of contortions at 400 mg/kg [60]. The mixture of 6α-hydroxy-7β-acetoxy-vouacapan-17β-oate methyl ester and 6α-acetoxy-7β-hydroxy-vouacapan-17β-oate methyl ester (i.p.) produced 76% of inhibition at 300 mg/kg [88].

So, we can propose that the observed effects of HEPpg must be due to the synergy of all these compounds. To reinforce this assumption, isolated vouacapan derivatives exhibited antinociceptive activity at concentrations greater than those of HEPpg used throughout this work (0.5 and 1 mg/kg) which produced 100% inhibition of writhes.

Conclusion

HEPpg was the extract with the highest anti-inflammatory and antinociceptive actions and comparison with literature data suggests a synergy of compounds due to higher antinociceptive activity with a lower dose than that observed with isolated vouacapan derivatives.

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References

1. Cruvinel WM, Mesquita DJ, Araújo JA, Catelan TT, de Souza AW, da Silva NP, Andrade LE. Immune system - part I. Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. Rev Bras Reumatol 2010;50:434-461.
2. Nathan C. Points of control in inflammation. Nature 2002;420:846-852.
3. Calder PC, Albers R, Antoine J-M, Blum S, Bourdet-Sicard R, Ferns GA, Folkerts G, Friedmann PS, Frost GS, Guarner F, Løvik M, Macfarlane S, Meye PD, Rabe L M't, Serafini M, van Eden W, van Loo J, Vas Dias W, Vidry S, Winklhofer-Roob BM, Zhao J. Inflammatory disease processes and interactions with nutrition. Br J Nutr 2009;101(1, Suppl):S1-45.
4. Dinarello CA. Anti-inflammatory Agents: Present and Future. Cell 2010;140:935-950.
5. Corrêa AD, Siqueira-Batista R, Quintas LEM. Plantas medicinais: do cultivo à terapêutica (7th ed.). Vozes Ed. Petrópolis, RJ, 2008:247.
6. Coimbra R. Notas de Fitoterapia. Catɑlogo dos dados principais sobre plantas utilizadas em medicina e farmɑcia. Araújo CS Ed. 1942;222.
7. Cruz G L. Livro verde das plantas medicinais e industriais do Brasil (1st ed.). Belo Horizonte, MG. 1965;2:779-780.
8. Velozo LS, Martino T, Vigliano MV, Pinto FA, Silva GP, Justo MG, Sabino KC, Coelho MG. Pterodon polygalaeflorus Essential oil modulates acute inflammation and B and T Lymphocyte activation. Am J Chin Med 2013;41(3):545-63.
9. Leal LKAM, Ferreira AAG, Bezerra GA, Matos FJA, Viana GSB.

Antinociceptive, anti-inflammatory and bronchodilatador activities of Brazilian medicinal plants containing coumarin: a comparative study. J Ethnopharmacol 2000;70:151-159.
10. Silva MCC, Gayer CRM, Lopes CS, Calixto NO, Reis PA, Passaes CPB, Paes MC, Dalmau SR, Sabino KCC, Todeschini AR, Coelho MGP. Acute and topic anti-edematogenic fractions isolated from the seeds of Pterodon pubescens. J Pharm Pharmacol 2004;55:135-141.
11. Coelho LP, Reis PA, Castro FL, Gayer CRM, Lopes CS, Silva MCC, Sabino KCC, Todeschini AR, Coelho MGP. Antinociceptive properties of ethanolic extract and fractions of Pterodon pubescens Benth. Seeds. J Ethnopharmacol 2005;98:109-116.
12. Calixto NO, Silva MCC, Gayer CRM, Coelho MGP, Paes MC, Todeschine, AR. Antiplatelet activity of geranylgeraniol isolated from Pterodon pubescens fruit oil is mediated by inhibition of Cyclooxygenase-1. Planta Med 2007;73:480-483.
13. Sabino KCC, Gayer CRM, Vaz LCA, Santos LRL, Felzeszwalb I, Coelho MGP. In vitro and in vivo toxicological studies of the Pterodon pubescens seed oil. Toxicol Lett 1999;108:27-35.
14. Coelho MGP, Marques PM, Gayer CRM, Vaz LCA, Neto JFN, Sabino KCC. Subacute toxicity evaluation of a hydroalcoholic extract of Pterodon pubescens seeds in mice with collagen-induced arthritis. J Ethnopharmacol 2001;77:159-164.
15. Coelho MG, Sabino KC, Dalmau SR. Immunomodulatory effects of sucupira (Pterodon pubescens) seed infusion on collagen-induced arthritis. Clin Exp Rheumatol 2004;22:213-218.
16. Zimmermann, M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109-110.
17. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Meth 1983;65:55-63.
18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131-138.
19. Selye H. Induction topical resistance to acute tissue injury; an experimental study with the granuloma pouch technique. Surg Clin North Am 1953;33:1417-1466.
20. Sedgwick AD, Moore AR, Al-Duaij AY. The immune response to pertussis in the 6-day air pouch: a model of chronic synovitis. Brit J Exp Pathol 1985;66:455-464.
21. Koster R, Anderson M, de Beer EJ. Acetic acid for analgesic screening. Fed Proc 1959;18:412-416.
22. Loro JF, del Rio I, Pérez-Santana L. Preliminary studies of analgesic and anti-inflammatory properties of Opuntia dillenii aqueous extract. J Ethnopharmacol 1999;67:213-218.
23. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Carol Stream, Illinois. 2001.
24. Fascio M, Mors WB, Gilbert B, Mahajan JR, Monteiro MB, Dos Santos Filho D, Vichnewsky W. Diterpenoids furans from Pterodon pubescens species. Phytochem 1976;15:201-203.
25. Nucci C, Mazzardo-Martins L, Stramosk J, Brethanha LC, Pizzolatti MG, Santos ARS, Martins DF. Oleaginous extract from the fruits Pterodon pubescens Benth induces antinociception in animal models of acute and chronic pain. J. Ethnopharmacol 2012;143:170-178.
26. Hoscheid J, Reinas A, Cortez DAG, Costa WF, Cardoso MLC. Determination by GC–MS-SIM of furanoditerpenes in Pterodon pubescens Benth.: Development and validation. Talanta 2012;100:372-376.
27. Cabral EC, Sevart L, Spindola HM, Coelho MB, Sousa IMO, Queiroz NCA, Foglio MA, Eberlina MN, Riverosc JM. Pterodon pubescens oil: characterization, certification of origin and quality control via mass spectrometry fingerprinting analysis. Phytochem Anal 2013;24:184-192.
28. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 2008;8:349-361.
29. Okoli CO, Akah PA, Nwafor SV. Anti-inflammatory activity of plants. J Nat Rem 2003;3:1-30.
30. Wink DA, Hines HB, Cheng RYS, Switzer CH, Flores-Santana W, Vitek MP, Ridnour LA, Colton CA. Nitric oxide and redox mechanisms in the immune response. J Leuk Biol 2011;89:873-891.
31. Guzik TJ, Korbut R, Adamek-Guzik T. Nitric oxide and superoxide in inflammation and immune regulation. J Physiol Pharmacol 2003;54:469-487.
32. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacol 2007;15:252-259.
33. Hofseth LJ. Nitric oxide as a target of complementary and alternative medicines to prevent and treat inflammation and cancer. Cancer Lett 2008;268:10-30.
34. Davis KL, Martin E, Turko IT, Murad F. Novel effects of nitric oxide. Ann Rev Pharmacol Toxicol 2001;41:203-236.
35. Reis J, Guan XQ, Kisselev AF, Papasian CJ, Qureshi AA, Morrison DC, Van Way III CW, Vogel SN, Qureshi N. LPS-induced formation of immunoproteasomes: TNF-α and nitric oxide production are regulated by altered composition of proteasome-active sites. Cell Biochem Biophys 2011;60:77-88.
36. Oh BK, Mun J, Seo HW, Ryu SY, Kim YS, Lee BH, Oh KS. Euonymus alatus extract attenuates LPS-induced NF-êB activation via IKKβ inhibition in RAW 264.7 cells. J Ethnopharmacol 2011;134:288-293.
37. Lii CK, Chen HW, Yun WT, Liu KL. Supressive effects of wild bitter gourd (Momordica charantia Linn. var. abbreviate ser.) fruit extracts on inflammatory responses in RAW264.7 macrophages. J Ethnopharmacol 2009;122:227-233.
38. Sedgwick AD, Sin YM, Edwards JCM, Willoughby DA. Increased inflammatory reactivity in newly formed lining tissue. J Pathol 1983;141:483-495.
39. Romano M, Faggioni R, Sironi M, Sacco S, Echtenacher B, Di Santo E, Salmona M, Ghezzi. Carrageenan-induced acute inflammation in the mouse air pouch synovial model. Role of tumour necrosis factor. Mediators Inflamm 1997;6:32-38.
40. Ellis L, Gilston V, Soo C, Morris C, Kidd B, Winyard P. Activation of the transcription factor NF-kb in the rat air pouch model of inflammation. Ann Rheum Dis 2000;59:303-307.
41. Woodfin A, Voisin MB, Beyrau M, Colom B, Caille D, Diapouli FM. The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. Nat Immunol 2011;12:761-769.
42. Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature 2001;413:203-210.
43. Collier HOJ, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Brit J Pharmacol 1968;32:295-310.
44. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev 2001;53:597-652.
45. Sani MHM, Zakaria ZA, Balan T, Teh LK, Salleh MZ. Antinociceptive activity of methanol extract of Muntingia calabura leaves and the mechanisms of action involved. Evid Based Complem Alternat Med 2012;2012:890361.
46. Ribeiro RA, Vale ML, Thomazz SM, Paschoalato ABP, Poole S, Ferreira SH, Cunha FQ. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol 2000;387:111-118.
47. Ikeda Y, Ueno A, Naraba H, Oh-Ishi S. Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. Life Sci 2001;69:2911-2919.
48. Campos AM, Silveira ER, Braz-Filho R, Teixeira TC. Diterpenoids from Pterodon polygalaeflorus. Phytochem

1994;36:403-406.
49. Sabino KCC, Castro FA, Oliveira JCR, Dalmau SRA, Coelho MG. Successful treatment of collagen-induced arthritis in mice with hydroalcohol extract of seeds of Pterodon pubescens. Phytother Res 1999;13:613-615.
50. Pereira MF, Simão TA, Dalmau SR, Albano RM, Coelho MGP, Sabino KCC. Pterodon pubescens seed extract induces the cell cycle arrest of leukemic cells by deregulating cyclin D1 and E2 mRNA levels. Oncol Lett 2010;1:533-536.
51. Pereira MF, Martino T, Dalmau SR, Albano RM, Ferezou J-P, Costa SS, Coelho MGP, Sabino KCC. Terpenic subfraction of Pterodon pubescens induces apoptosis of K562 leukemic cells by modulating gene expression. Oncol Rep 2011;25:215-221.
52. Mahajan JR, Monteiro MB. New diterpenoids from Pterodon emarginatus Vog. An Acad Brasil Ciênc 1970;42(Suplem): 03-106.
53. Mahajan JR, Monteiro MB. New diterpenoids from Pterodon emarginatus Vog.. J Chem Soc Perkin Trans 1973;4:520-525.
54. Marques DD, Machado MIL, De Carvalho MG, Meleira LAC, Braz-Filho R. Isoflavonoids and triterpenoids isolated from Pterodon polygalaeflorus. J Braz Chem Soc 1998;9:295-301.
55. Pellegrino J. Protection against human Schistosome cercariae. Exp Parasitol 1967;21:112-131.
56. Mors WB, Dos Santos Filho F, Monteiro HJ, Gilbert B, Pellegrino J. Chemoprophylatic agent in schistosomiasis: 14,15-epoxygeranylgeraniol. Science 1967;157:950-951.
57. Almeida MEL, Gottlieb OR. Further isoflavones from Pterodon apparicioi. Phytochem 1975;14:2714.
58. Carvalho JCT, Sertie JAA, Barbosa MVJ, Patricio KCM, Caputo LRG, Sarti SJ, Ferreira LP, Bastos JK. Anti-inflammatory activity of the crude extract from the frutis of Pterodon emarginatus Vog. J Ethnopharmacol 1999;64:127-133.
59. Arriaga AMC, Castro MAB, Silveira ER, Braz-Filho R. Further diterpenoids isolated from Pterodon polygalaeflorus. J Braz Chem Soc 2000;11:187-190.
60. Galceran CB, Sertie JAA, Lima CS, Carvalho JCT. Anti-inflammatory and of 6a,7b–dihydroxy-vouacapan-17b-oic acid isolated analgesic effects from Pterodon emarginatus Vog. Fruits. Inflammopharmacol 2011;19:139-143.
61. Leonhardt V, Leal-Cardoso JH, Lahlou S, Albuquerque AAC, Porto RS, Celedônio NR, Oliveira AC, Pereira RF, Silva LP, Garcia-Teófilo TMN, Silva APFS, Magalhães PJC, Duarte GP, Coelho-de-Souza NA. Antispasmodic effects of essential oil of Pterodon polygalaeflorus and its main constituent β-caryophyllene on rat isolated ileum. Fund Clin Pharmacol 2010;24:749-758.
62. Dutra RC, Pittella F, Dittz D, Marcon R, Pimenta DS, Lopes MTP, Raposo NRB. Chemical composition and cytotoxicity activity of the essential oil of Pterodon emarginatus. Braz J Pharmacog 2012;22:971-978.
63. Alves SF, Borges LL, de Paula JAM, Vieira RF, Ferri PH, do Couto RO, de Paula JR, Bara MTF. Chemical variability of the essential oils from fruits of Pterodon emarginatus in the Brazilian Cerrado. Braz J Pharmacog 2013;23:224-229.
64. Braz-Filho R, Gottlieb OR. The isoflavones from Pterodon pubescens. Phytochem 1971;10:2835-3836.
65. Spindola HM, Carvalho JE, Ruiz ALTG, Rodrigues AFR, Denny C, Sousa IMO, Tamashiro JY, Foglio MA. Furanoditerpenes from Pterodon pubescens Benth. with selective in vitro anticancer activity for prostate cell line. J Braz Chem Soc 2009;20:569-575.
66. Nunan EA, Carvalho MG, Piló-Veloso D, Turchetti-Maia RMM, Ferreira-Alves DL. Furane diterpenes with anti- and proinflammatory activity. Rev Bras Pesq Méd Biol 1982;15:450-451.
67. Duarte IDG, Ferreira-Alves DL, Veloso DP, Nakamura-Craig M. Evidence of the involvement of biogenic amines in the antinociceptive effect of a vouacapan extracted from Pterodon polygalaeflorus Benth.. J Ethnopharmacol 1996;55:13-18.
68. Lorenzi H, Matos FJA. Plantas medicinais no Brasil: nativas e exóticas. Instituto Plantarum Ed., São Paulo, pp. 512. Ed. Instituto Platarum de Estudos da Flora Ltda, Nova Odessa, São Paulo, 2002:512.
69. Spindola HM, Serva J, Denny C, Rodrigues RAF, Eberli MN, Cabra E, Sousa IMO, Tamashiro JY, Carvalho JE, Foglio MA. Antinociceptive effect of geranylgeraniol and 6a,7b-dihydroxyvouacapan-17b-oate methyl ester isolated from Pterodon pubescens Benth. BMC Pharmacology 2010;10:1-10.
70. Wu L, Wang G, Tang S, Long G, Yin T. Protection of endothelial cells inhibition of neointimal hyperplasia by β-elemene in an injured artery. Cardiovasc Drugs Ther 2011;25:233-242.
71. Chen W, Lu Y, Wu J,Gao M, Wang A, Xu B. Beta-elemene inhibits melanoma growth and metastasis via suppressing vascular endothelial growth factor-mediated angiogenesis. Cancer Chemother Pharmacol 2011;67:799-808.
72. Li QQ, Wang G, Zhang M, Cuff CF, Huang L, Reed E. Betaelemene, a novel plant-derived antineoplastic agent, increases cisplatin chemosensitivity of lung tumor cells by triggering apoptosis. Oncol Rep 2009;22:161-170.
73. Lee RX, Li OQ, Reed E. β-Elemene effectively suppresses the growth and survival of both platinum-sensitive and -resistant ovarian tumor cells. Anticancer Res 2012;32:3103-3114.
74. Cho JA, Chang HJ, Lee S-K, Kim H-J, Hwang J-K, Chun HS. Amelioration of dextran sulphate sodium-induced colitis in mice by oral administration of β-caryophyllene, a sesquiterpene. Life Sci 2007;80:932-939.
75. Kubo I, Chaudhuri SK, Kubo Y, Sanchez Y, Ogura T, Saito T, Ishikawa H. Cytotoxic and antioxidative sesquiterpenoids from Heterotheca inuloides. Planta Med 1996;62:427-430.
76. Pichette A, Larouche P-L, Lebrun M, Legault J. Composition and antibacterial activity of Abies balsamea essential oil. Phytother Res 2006;20:371-373.
77. Singh G, Marimuthu P, de Heluani C S, Catalan CA. Antioxidant and biocidal activities of Carum nigrum (seed) essential oil, oleoresin, and their selected components. J Agric Food Chem 2006;54:174-181.
78. Ghelardini C, Galeotti N, Di Cesare M-L, Mazzanti G, Bartolini A. Local anaesthetic activity of b-caryophyllene. Farmaco 2001;56:387-389.
79. Leonhardt V, Leal-Cardoso JH, Lahlou S, Albuquerque AAC, Porto RS, Celedônio NR, Li QQ, Wang G, Huang F, Banda M, Reed E. Antineoplastic effect of beta-elemene on prostate cancer cells and other types of solid tumour cells. J Pharm Pharmacol 2010b;62:1018-1027.
80. Chavan MJ, Wakte PS, Shinde DB. Analgesic and anti-inflammatory activity of Caryophyllene oxide from Annona squamosa L. bark. Phytomedicine 2010;17:149-151.
81. Rogerio AP, Andrade EL, Leite AFP, Figueiredo C.P., Calixto JB. Preventive and therapeutic anti-inflammatory properties of the sesquiterpene α-humulene in experimental airways allergic inflammation. Br J Pharmacol 2009;158:1074-1087.
82. Ziaei A, Ramezani M, Wright L, Paetz C, Schneider B, Amirghofran Z. Identification of spathulenol in Salvia mirzayanii and the immunomodulatory effects. Phytother Res 2011;25:557-562.
83. Meigs TE, Sherwood SW, Simoni RD. Farnesyl acetate, a derivative of an isoprenoid of the mevalonate pathway, inhibits DNA replication in hamster and human cells. Exp Cell Res 1995;219:461-470.
84. Khan R, Sultana S. Farnesol attenuates 1,2-dimethylhydrazine induced oxidative stress, inflammation

and apoptotic responses in the colon of Wistar rats. Chem Biol Interact 2011;192:193-200.
85. de Oliveira Júnior WM, Benedito RB, Pereira WB, de Arruda Torres P, Ramos CA, Costa JP, da Rocha Tomé A, de Sousa DP, de Freitas RM, de Fatima Formiga Melo Diniz M, de Almeida RN. Farnesol: antinociceptive effect and histopathological analysis of the striatum and hippocampus of mice. Fundam Clin Pharmacol 2012;27:419-426.
86. Duarte IDG, Ferreira-Alves DL, Veloso DP, Nakamura-Craig M. Possible participation of endogenous opioid peptides on the mechanism involved in analgesia induced by vouacapan. Life Sci 1992;50:891-897.
87. Spindola HM, Servat L, Rodrigues RAF, Sousa IMO, Carvalho JE, Foglio MA. Geranylgeraniol and 6α,7β-dihydroxyvouacapan-17β-oate methyl ester isolated from Pterodon pubescens Benth.: Further investigation on the antinociceptive mechanisms of action. Eur J Pharmacol 2011;656:45-51.
88. Servat L, Spindola HM, Rodrigues, RAF, Sousa IMO, Ruiz ALTG, de Carvalho JE, Foglio, MA. Pterodon pubescens Benth: stability study of microencapsulated extract and isolated compounds monitored by antinociceptive assays. J Braz Chem Soc 2012;23:1201-1208.

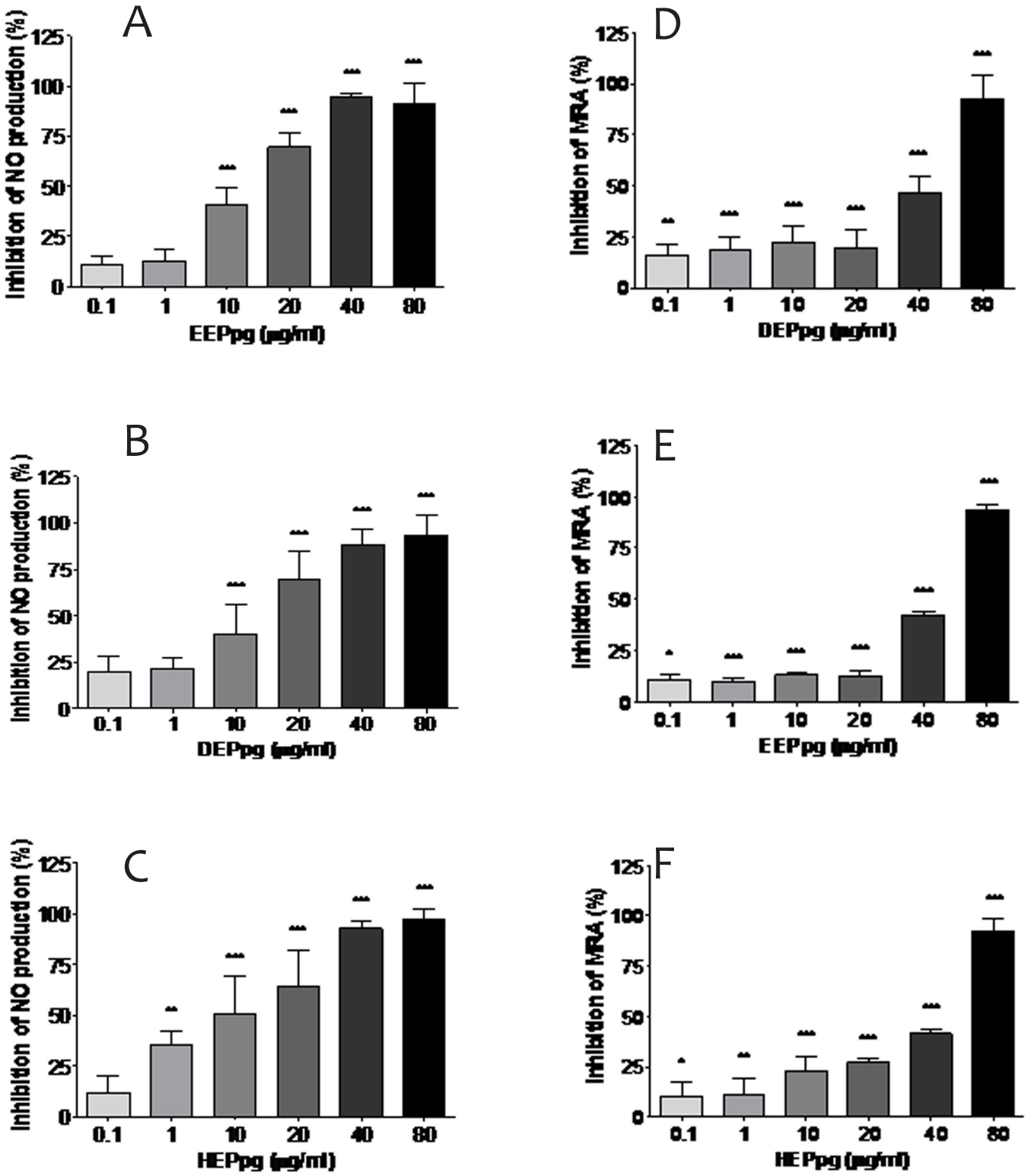

Figure 1: Effects of EEPpg (A, D), DEPpg (B,E) and HEPpg (C, F) on the nitric oxide production (A-C) and cytotoxicity (D-F) of macrophage RAW 264.7 cell line. RAW 264.7 cells (5 x 10^5 cells/ml) were stimulated with LPS in the absence (control) and presence of different concentrations of extracts for 24 h. The cytotoxicity of the samples was assessed by MTT assay, and the results expressed as percentage of mitochondrial reducing activity (MRA) in relation to the control culture.

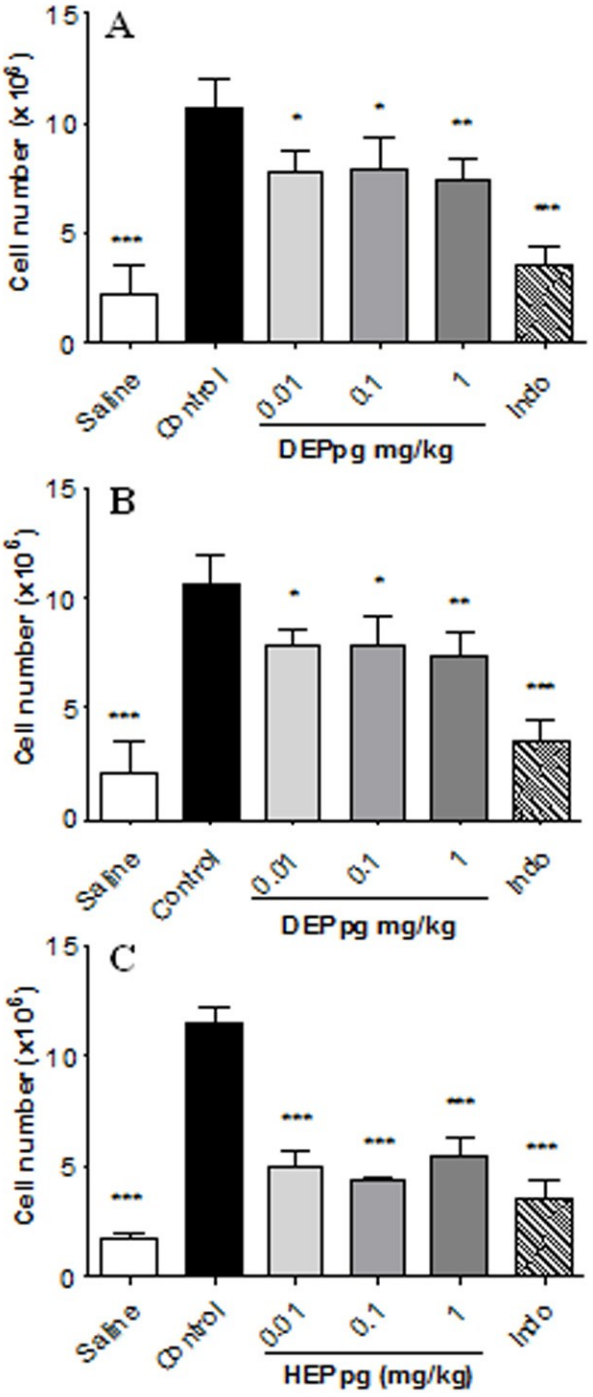

Figure 2: Effects of EEPpg (A), DEPpg (B) and HEPpg on the total number of leucocytes in the exudate of the carrageenan-induced air-pouch model. Inflammation was induced by injection of 1 ml carrageenan 1% in the air pouch. SW male mice (n = 5/group) were treated (p.o.) with the vehicle (control), Indomethacin (Indo, 10 mg/kg) or with different doses of extracts prior to injection of carrageenan. A control group received only saline on the cavity (saline). The results express mean ± SD of three experiments. *p<0.05, **p<0.01, and ***p < 0.001 in relation to control group by Tukey's test, after one-way ANOVA.

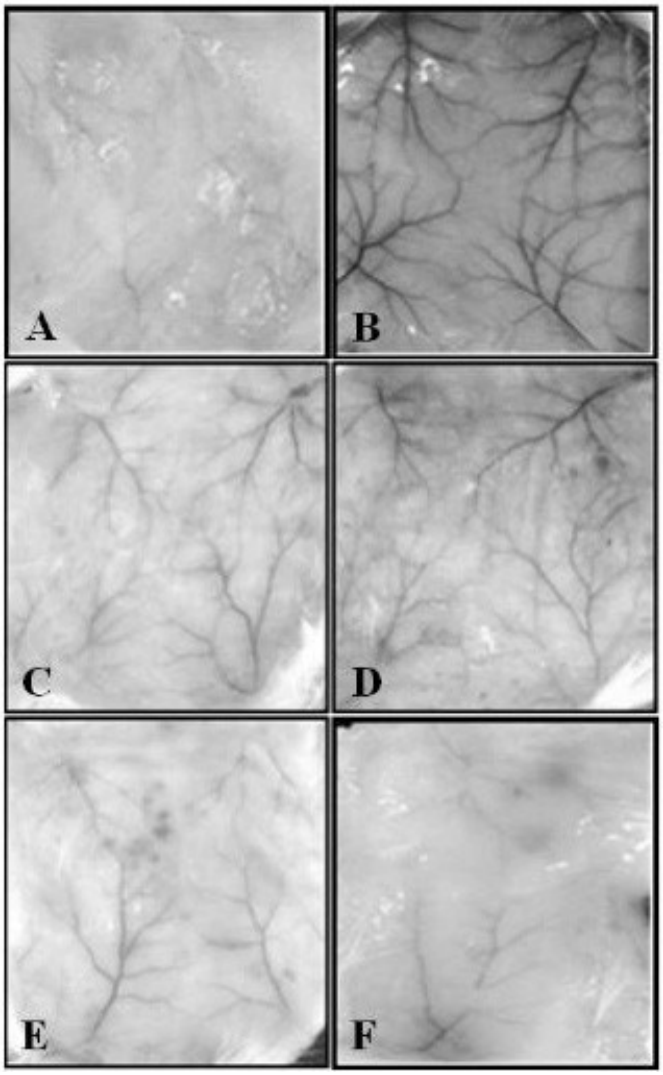

Figure 3. Macroscopical evaluation of pouches tissues after treatment with different *P. polygalaeflorus* extracts. (A) Animals inoculated with saline; (B) Control group with carrageenan; (C) EEPpg 1 mg/kg; (D) DEPpg 1 mg/kg; (E) HEPpg 1 mg/kg; (F) Indomethacin 10 mg/kg. Photographies are representative of three experiments.

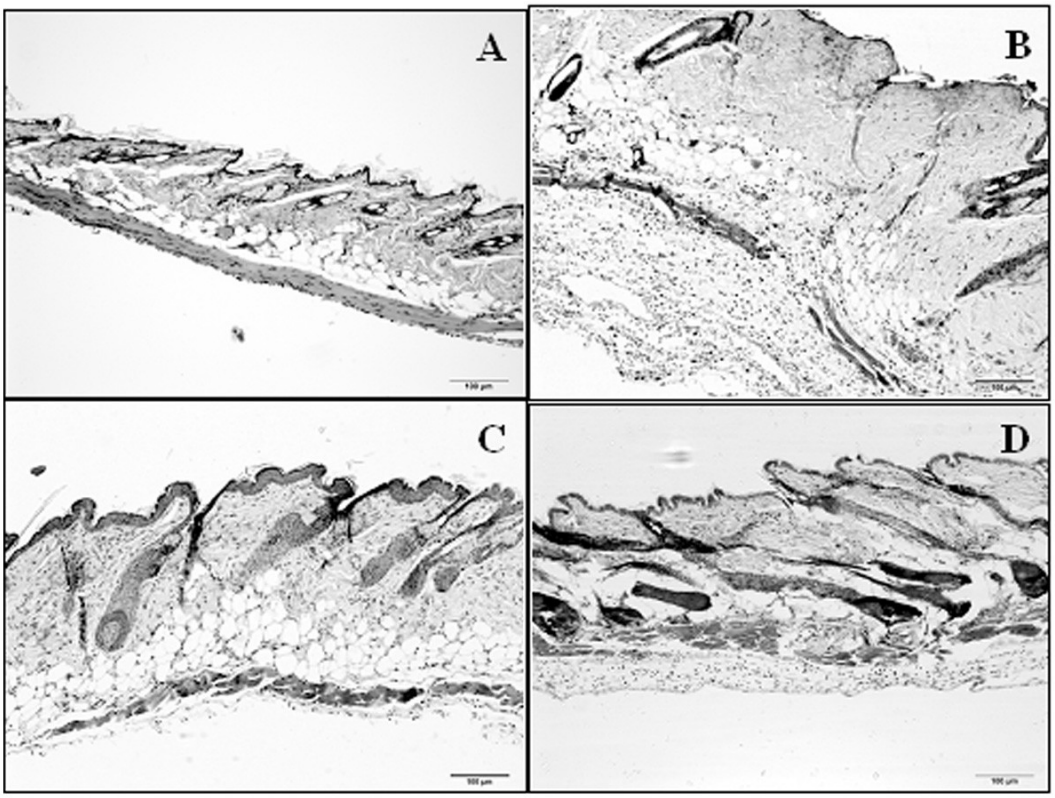

Figure 4: Photomicrographs of air pouch tissues from mice treated with HEPpg compared with control group. (A) Animal inoculated with saline; (B) Control group with carrageenan; (C) Indomethacin 10 mg/kg; (D) HEPpg 1 mg/kg. Images show histological changes of representative animals of each group. H&E-stained 5 ìm sections (x 100 magnification).

Group/dose	EEPpg		DEPpg		HEPpg	
	Writhing ± SD	% inhibition	Writhing ± SD	% inhibition	Writhing ± SD	% inhibition
Control	41.9 ± 6.5	-	41.7 ± 6.5	-	41.7 ± 6.5	-
AAS 100 mg/kg	23.1 ± 4.9 [a]	44.6	24.0 ± 3.5[a]	42.4	22.9 ± 4.5 [a]	45.0
0.01 mg/kg	-	-	-	-	25.0 ± 4.7 [a]	40.1
0.05 mg/kg	-	-	22.7 ± 6.7 [a]	45.8	31.2 ± 9.9 [a]	25.3
0.1 mg/kg	22.0 ± 3.7 [a]	47.4	22.0 ± 3.7 [a]	47.4	30.7 ± 1.7 [a]	26.5
0.5 mg/kg	32.5 ± 1.7 [b]	22.2	24.1 ± 9.4 [a]	42.3	0 ± 0 [a]	100.0
1 mg/kg	24.7 ± 8.4 [a]	40.9	24.7 ± 8.3 [a]	40.9	0 ± 0 [a]	100.0
5 mg/kg	28.6 ± 7.7 [a]	31.5	11.7 ± 6.9 [a]	71.9	-	-

Table 1: Antinociceptive potential of different *P. polygalaeflorus* extracts on the acetic acid-induced writhing test. Values are mean ± SD (n=6). [a]p<0.001 e [b]p<0.01 in relation to control group (one-way ANOVA followed by Tukey's test).

Substance	EEPpg		DEPpg		HEPpg	
	R.T.	%	R.T.	%	R.T.	%
copaene[1]	-	-	-	-	27.19	1.01
β-elemene[1]	27.95	13.42	27.95	10.17	27.95	10.10
caryophyllene[1]	29.09	15.65	29.09	15.93	29.09	14.01
α-humulene[1]	30.56	1.29	30.56	1.38	30.56	1.61
germacrene D[1]	31.75	10.05	31.75	8.78	-	-
germacrene B[1]	32.42	2.37	32.42	1.81	-	-
Spathulenol[2]	-	-	35.75	1.44	35.76	4.72
caryophyllene oxide[2]	-	-	35.94	1.12	35.96	12.21
iso-spathulenol[2]	-	-	-	-	39.48	1.09
Farnesol[2]	41.36	2.15	41.36	2.10	41.36	5.04
farnesyl acetate[2]	-	-	-	-	45.64	1.18
6α-hydroxy-7β-acetoxy vouacapan-14(17)-ene[3]	72.50	2.12	72.49	1.20	72.51	1.10
methyl-6α,7β-dihydroxy vouacapan-17β-oate[3]	73.02	3.54	73.02	4.25	73.04	7.18
6α-acetoxy-7β-hydroxy vouacapan[3]	73.85	10.12	73.86	9.97	73.86	6.09
6α,7β-diacetoxyvouacapan[3]	74.80	0.80	-	-	-	-
methyl-7β-acetoxy-6α-hydroxyvouacapan-17β-oate[3]	75.09	7.55	75.09	8.01	75.10	7.79
methyl-6α-acetoxy-7β-hydroxyvouacapan-17β-oate[3]	75.92	17.60	75.93	25.67	75.94	17.20

Table 2: Chemical composition of different *P. polygalaeflorus* extracts. Sesquiterpene hydrocarbons[1], oxygenated sesquiterpenes[2], and furan diterpenes[3].